CYTOLOGICAL EVIDENCE OF INTROGRESSION BETWEEN *DROSOPHILA* SPECIES IN PANAMA[1]

SARAH BEDICHEK PIPKIN[2]

ABSTRACT

Cytological evidence of introgression in the classical sense of Anderson (1949) and Stebbins (1950) has been presented for the sibling species *Drosophila metzii* and *Drosophila pellewae* in a population of the Isthmus of Darien, Panama. Salivary chromosome karyotypes of hybrids between the two species show multiple translocations involving the four large autosomes. A strain of *D. metzii* collected from a mixed population of the two species near El Real, Darien, Panama, displays both heterozygous and homozygous translocation figures and regions of asynapsis in salivary chromosomes of third instar larvae. A heterozygous translocation figure is seen in some salivary gland cells of larvae of the Darien strain of *D. pellewae*. Observations of variations in length of chromosomes in different individuals of the Darien *D. metzii* strain suggest restructuring arising presumably from crossing over in progenitors heterozygous for multiple translocations.

The requirements necessary for introgressive hybridization outlined by Anderson (1949) and Stebbins (1950: 251–297) are fulfilled by the relationship between the sibling species *Drosophila metzii* and *Drosophila pellewae*. Each species has a characteristic range with a region of overlap in the Isthmus of Darien. *Drosophila pellewae* has been collected from Barro Colorado Island, Canal Zone, where it is extremely rare, to Río Raposo, Colombia, where it is moderately common (Pipkin & Heed, 1964). In the north, *D. metzii* has been collected at Sonte Comapun (near Vera Cruz, Mexico) by Fabergé (personal communication), at Turrialba, Costa Rica (Pipkin, 1965), and San Salvador, El Salvador (Heed & Wheeler, 1957), and to the south as far as Santa Marta on the northern coast of Colombia (Heed, personal communication). Mixed populations of these siblings occur in forests, feeding over the same fallen fruit or flowers. Pipkin (1968*a*) has presented evidence that the species do cross in nature and that a specific character, white carina color, has been transferred from *D. metzii* into populations of *D. pellewae*, where an obscure carina color polymorphism is seen both in the Darien and Río Raposo populations. Although 280 individuals consisting of 60% *D. pellewae*, 40% *D. metzii* were collected from a single unusually large population in the Darien in 1961, gene exchange between the two species is greatly reduced, owing partly to translocations involving the four major autosomes (Pipkin, 1968*a*). A primitive karyotype consisting of five pairs of rod-shaped and one pair of dot-shaped chromosomes, characterizes both species according to analysis of larval ganglionic cells of strains originating

---

[1] Supported by NSF Grant GB-8770 and NIH Grant 1R 101 GM 18409-01.

The author is deeply indebted to Mr. Eric Schabtach, formerly of The Johns Hopkins University, now of The University of Oregon, Eugene, Oregon, not only for making the photographs used in this paper but also many other study photographs; to Dr. H. Ursprung, formerly of The Johns Hopkins University, for his generous permission to use the Zeiss photomicroscope; to Mr. L. M. Powers, for culturing *Drosophila metzii* and *Drosophila pellewae*.

[2] Post Office Box 138, Howard University, Washington, D. C. 20001.

ANN. MISSOURI BOT. GARD. 59: 417–434.

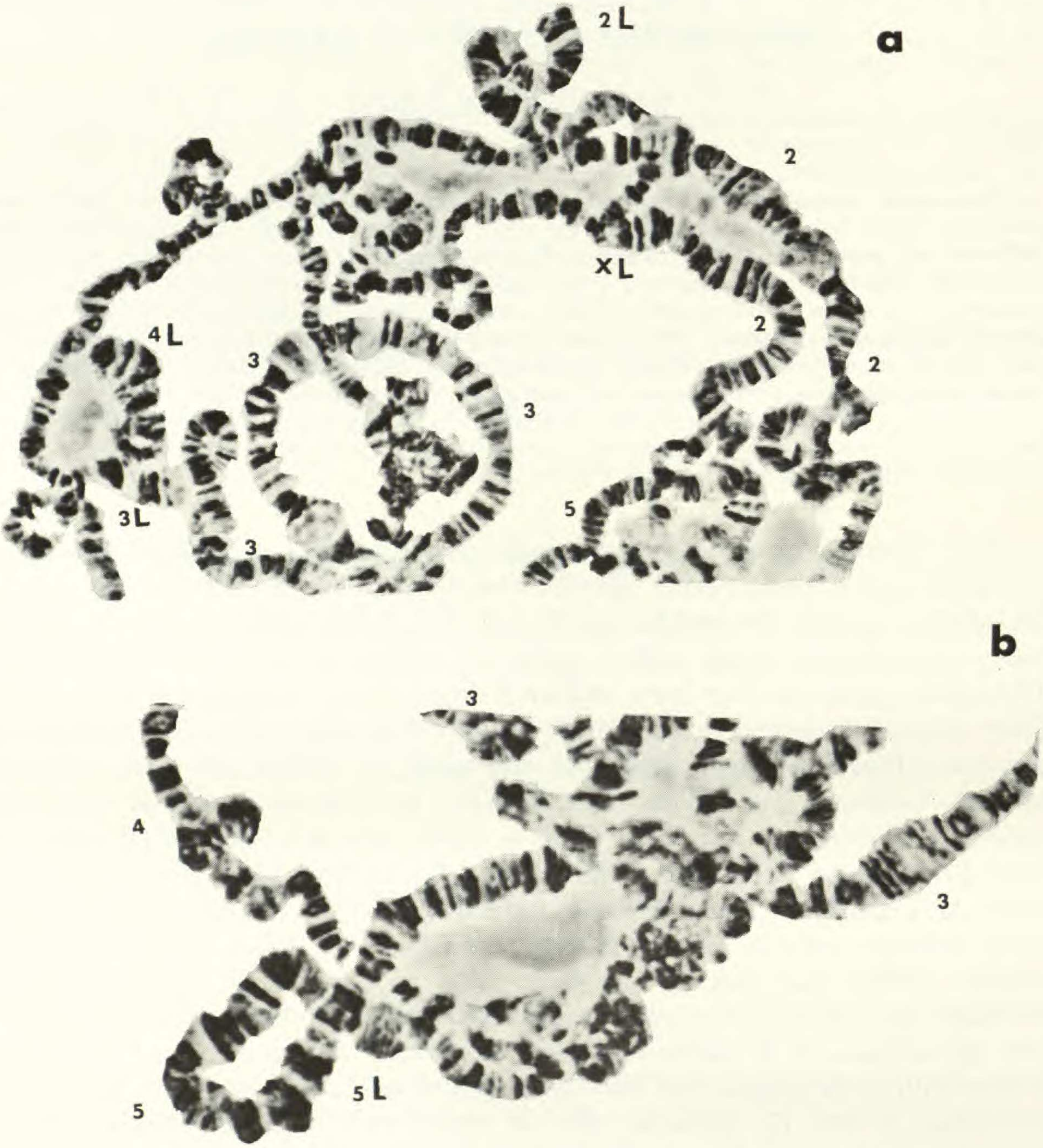

Figure 1. Photograph of salivary chromosomes of *Drosophila metzii/D. pellewae* hybrid. — A. Upper half of cell 1. — B. Lower half of cell 1. — One of the paired homologs of the distal end of chromosome 5 in Fig. 1B is joined to the unpaired homolog of another chromosome. Homologs of chromosome 4 are largely unpaired. The hybrid is heterozygous for a large paracentric inversion in chromosome 3.

outside the region of overlap (Pipkin & Heed, 1964). Salivary chromosome maps of nine members of the *tripunctata* species group, including the Barro Colorado Island strain of *D. metzii* have been made by Kastritsis (1966). The present paper will offer cytological evidence of introgression both from *pellewae* into *metzii* and from *metzii* into *pellewae* and evidence from octanol dehydrogenase isozyme patterns that the two species have nevertheless remained distinct in the Isthmus of Darien, Panama.

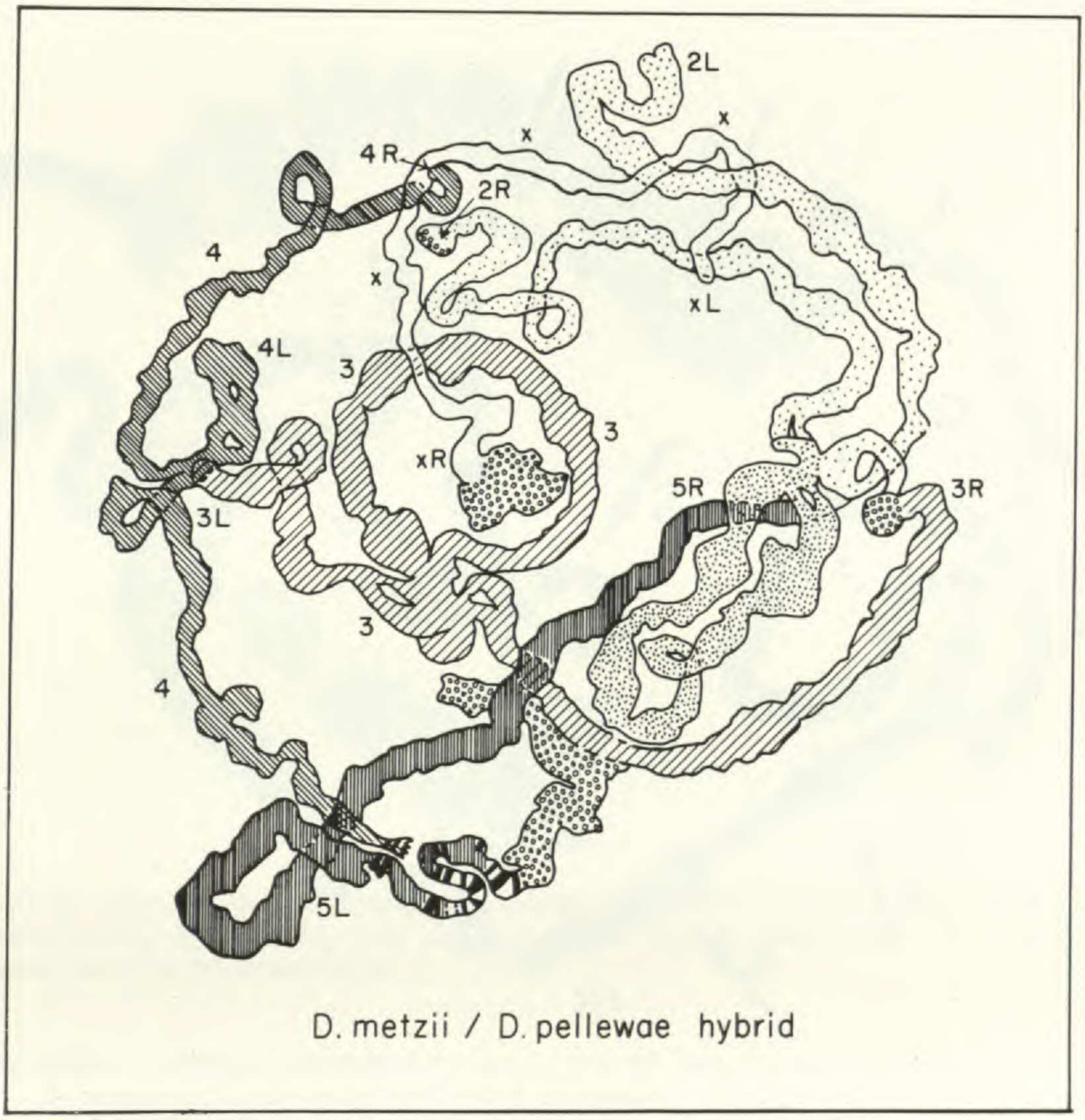

FIGURE 2. Diagram of chromosomes shown in Fig. 1.

MATERIALS AND METHODS

A description of the collection localities and numbers of founders of *Drosophila pellewae* from Barro Colorado Island, and Darien, Panama; Río Raposo (near Buenaventura), Colombia; and of the strains of *D. metzii* from Barro Colorado Island, Darien, and Almirante, Panama, and from Trinidad, West Indies, has been given by Pipkin (1968*a*). Temporary aceto-orcein lactic acid smears of third instar larvae were prepared according to a modified method of Wasserman (1954) described by Pipkin (1968*a*). Photographs of salivary gland chromosomes were made at varying focal depths using a Zeiss photomicroscope with phase contrast lighting at The Johns Hopkins University by Eric Schabtach, now of the Department of Biology, The University of Oregon.

RESULTS

1. Somatic pairing between homologs of salivary gland chromosomes of *D. metzii/D. pellewae* hybrids.

Pairing is usually close between homologous chromosomes of salivary gland cells of hybrids between *Drosophila metzii* and *D. pellewae*, affording an idea of the gross differences in banding sequence which distinguish these species. In hybrids using any of several strains of each species, complex translocation figures involving chromosomes 2, 3, 4, and 5 are seen (Pipkin, 1968*a*). The X chromo-

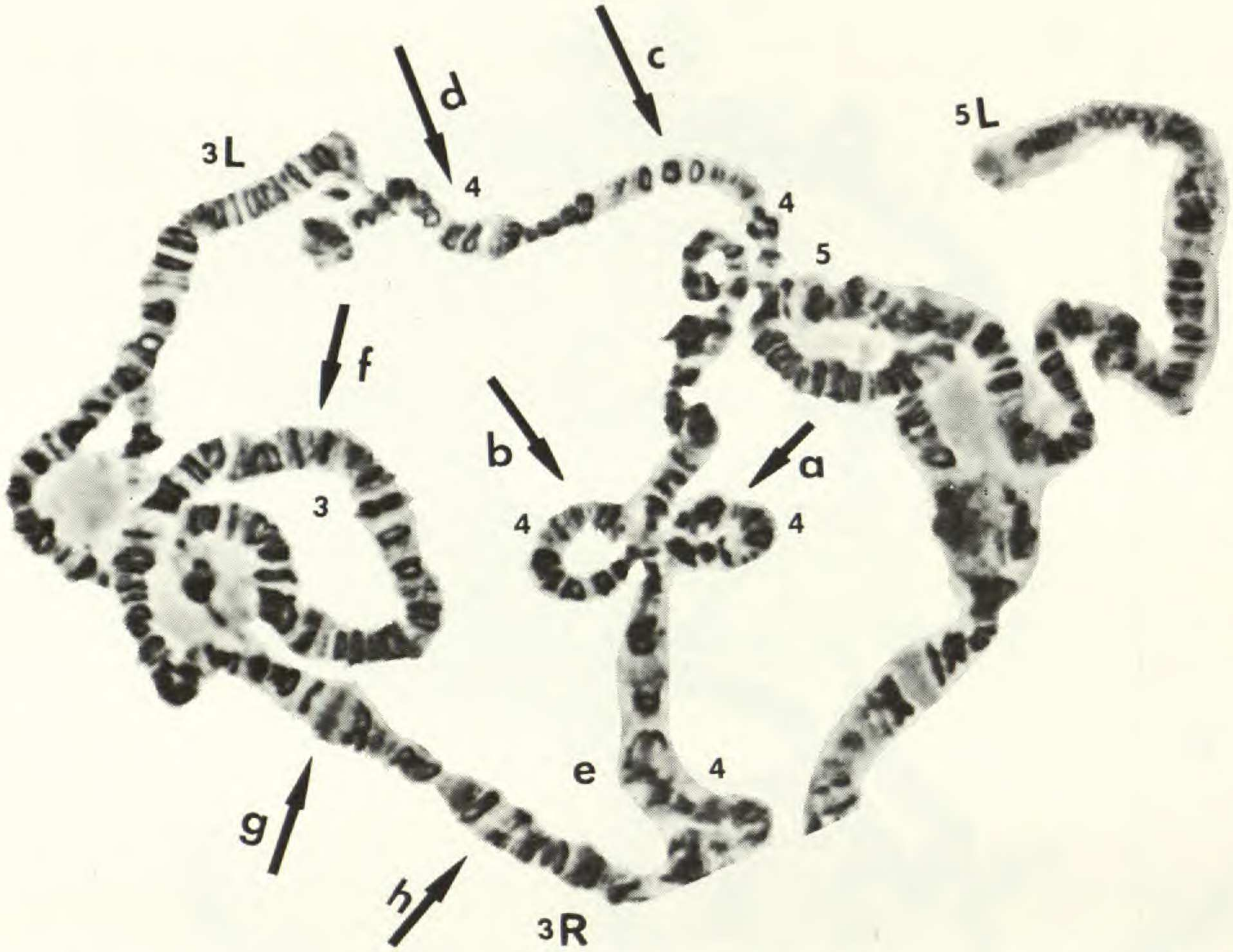

FIGURE 3. Photograph of part of cell 2 of a *Drosophila metzii*/*D. pellewae* hybrid showing at a and b heterozygous overlapping inversions figure in chromosome 4; c and d unpaired homolog of chromosome 4; e region of chromosome 4 where homologs are poorly paired; f a large heterozygous inversion figure in chromosome 3; g and h a region of poor pairing of chromosome 3.

some and short chromosome 6 are always free from the translocations. Most cytological work on species hybrids has been done using offspring of females of the Almirante strain of *metzii* and males of the Barro Colorado Island strain of *pellewae*. Photographs of the two halves of the cell of such a species hybrid appear in Figure 1A and B, together with an explanatory diagram of the entire cell in Figure 2. The X chromosome is free from the translocations involving the autosomes; its banding sequence resembles that of the X chromosome of *D. metzii* strain of Barro Colorado Island according to the map of Kastritsis. The banding sequences of the ends only of autosomes have been completely identified; these are indicated in the photographs (Fig. 1A,B) and diagram (Fig. 2). Some of the translocation interchanges have been broken in the cell shown in Fig. 1A and B, but certain features demonstrate prominent differences in banding patterns of the two species. The species hybrid is heterozygous for a large paracentric inversion in chromosome 3 which appears to be associated with the other chromosomes only at its heterochromatic end. Other cells of the same hybrid examined suggest that an interchange connecting

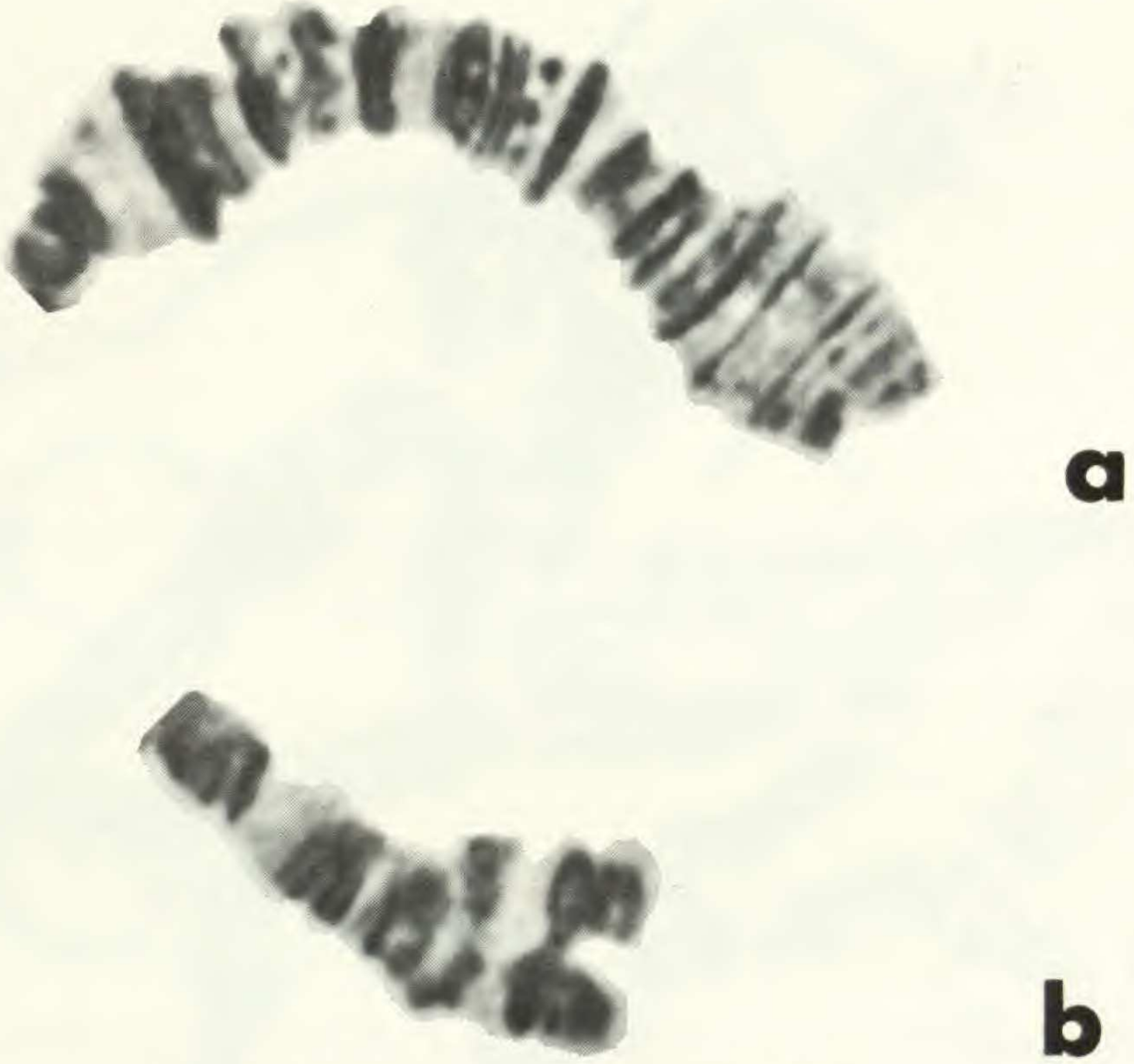

FIGURE 4. Chromosomes of *Drosophila metzii/D. pellewae* hybrid. — A. Distal ends of paired chromosome 5 homologs, with one end broken during squashing. — B. Poorly paired distal tips of chromosome 2 homologs.

chromosome 3 to the unidentified chromosome loop, which is heavily stippled in the diagram of Figure 2, has been broken by squashing the cell. The homologs of chromosome 4 are largely unpaired in Figure 1A,B, although at least a portion of the banding sequence of the two homologs is homosequential. More complete synapsis of the chromosome 4 homologs can be seen in another cell (Fig. 3) where an overlapping inversions figure is apparent. Even in the cell shown in Figure 3, the homologs of a portion of the distal region of chromosome 4 remain unpaired (indicated on the photograph by arrows at c, d), and homologs of the proximal end of this chromosome at e are poorly paired. In the *metzii/pellewae* hybrid of Figure 1B, the distal end of chromosome 5 is attached to the proximal part of another chromosome in one of the homologs. In another preparation (Fig. 4a) this interchange has been broken by squashing so that the distal end of one of the paired chromosome 5 homologs appears a little shorter than its partner. In the karyotype of a *metzii/pellewae* hybrid shown in Figure 5, chromosome 2 shows a short heterozygous inversion figure marked with an arrow at a and overlapping inversions at b before it enters a translocation cross-shaped figure at c. These inversion and translocation interchanges have been broken in the cell shown in Figure 1A and in the diagram, Figure 2. In hybrids between *metzii* and *pellewae*, homologs of the distal tip of chromosome 2 are often unpaired or poorly so, as in Figures 1A, 4B and 5.

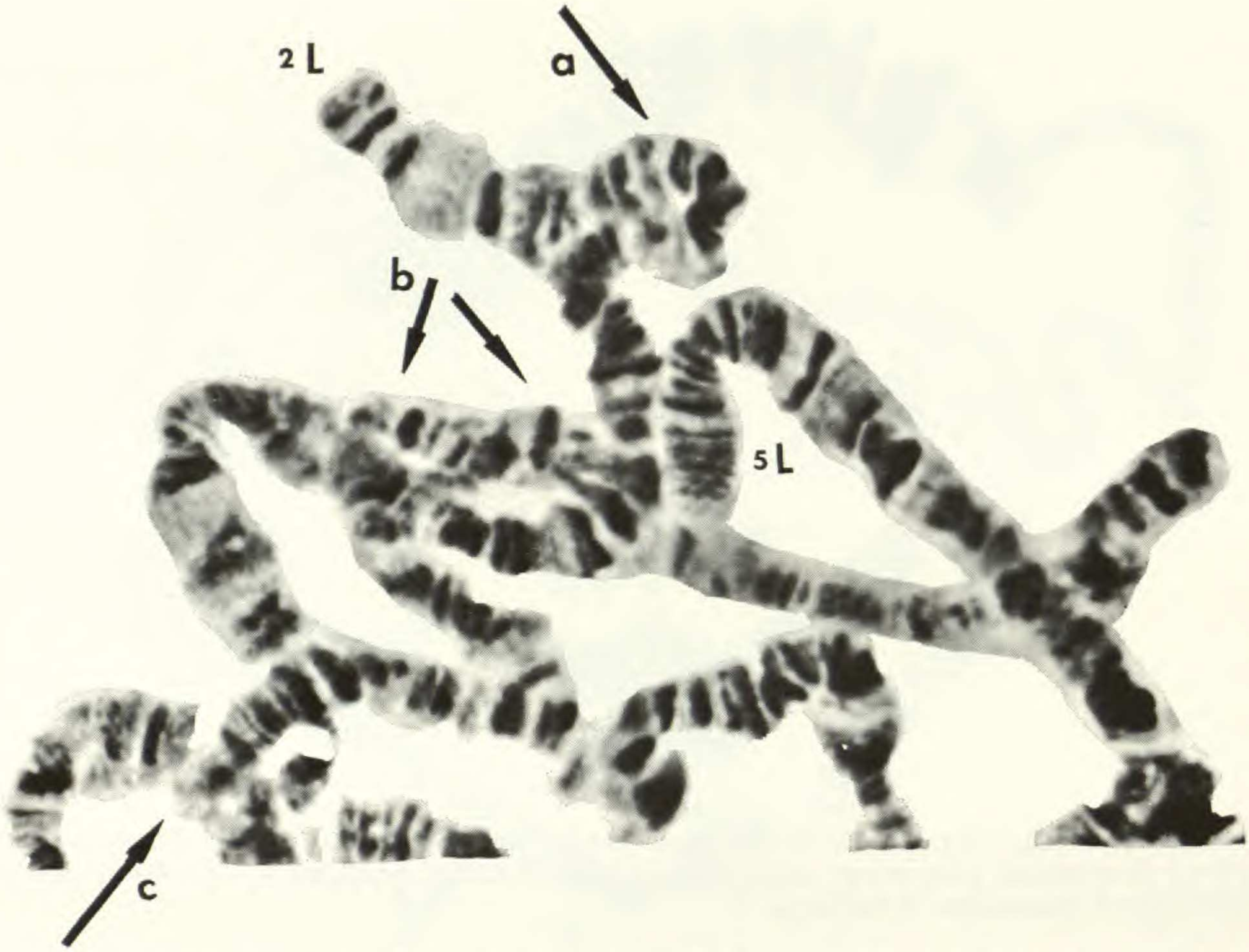

FIGURE 5. Photograph of part of cell 3 of a *Drosophila metzii/D. pellewae* hybrid showing a short inversion, a, in chromosome 2; b, a heterozygous overlapping inversions figure; c, an interchange of a heterozygous translocation figure.

2. Evidence of transfer of portions of the *Drosophila pellewae* genome into that of *D. metzii* in the Darien population.

A number of cells prepared from the Darien strain of *Drosophila metzii* showed six paired homologs of lengths similar to those observed by Kastritsis (1966) in the Barro Colorado Island strain of *D. metzii*, as in the photograph (Fig. 6) and diagram (Fig. 7). A large mass of heterochromatin usually present at the proximal portion of the X chromosome has broken away in this cell. The karyotype of this cell, where the relative lengths of homologs may be represented as $2 > 3 \sim X > 4 > 5 > 6$, is arbitrarily designated as standard. Several different heterozygous translocation figures appearing to involve two non-homologous chromosomes only have been seen in larvae of the Darien strain of *metzii*. For example, Figure 8 shows a photograph of a cell of a larva heterozygous for a translocation involving chromosomes 2 and 4. The banding pattern of the portion of the chromosomes outside the region of inversions is closely similar to that of parts of chromosomes 2 and 4 of the *D. metzii* map of Kastritsis (1966). The overlapping inversions observed in the *metzii/pellewae* hybrid of Figure 4 resemble those of Figure 8 and suggest that

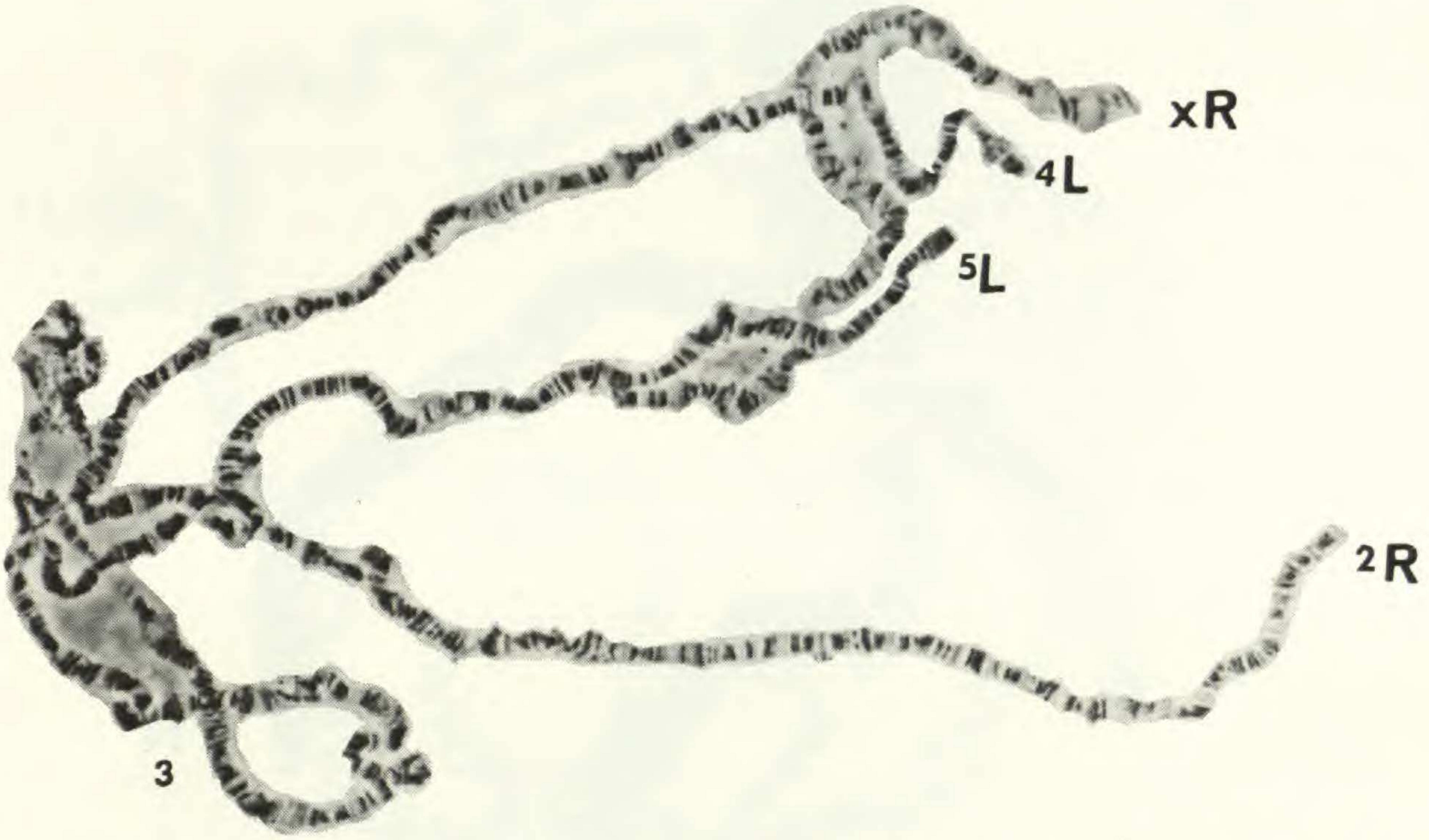

FIGURE 6. Photograph of a cell of an individual of the Darien strain of *Drosophila metzii* with a standard karyotype in which the relative chromosome lengths are 2 > X ~ 3 > 4 > 5.

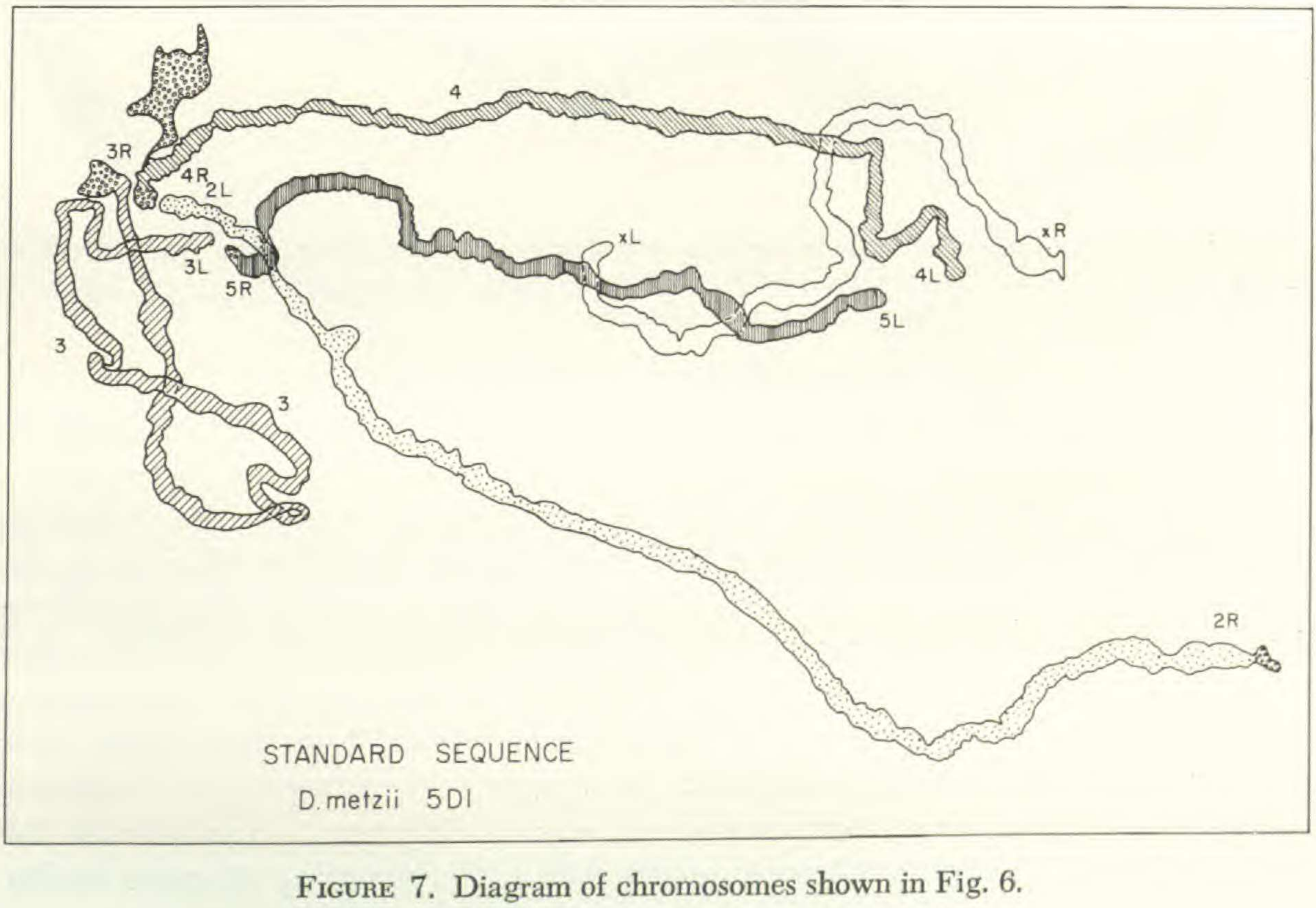

FIGURE 7. Diagram of chromosomes shown in Fig. 6.

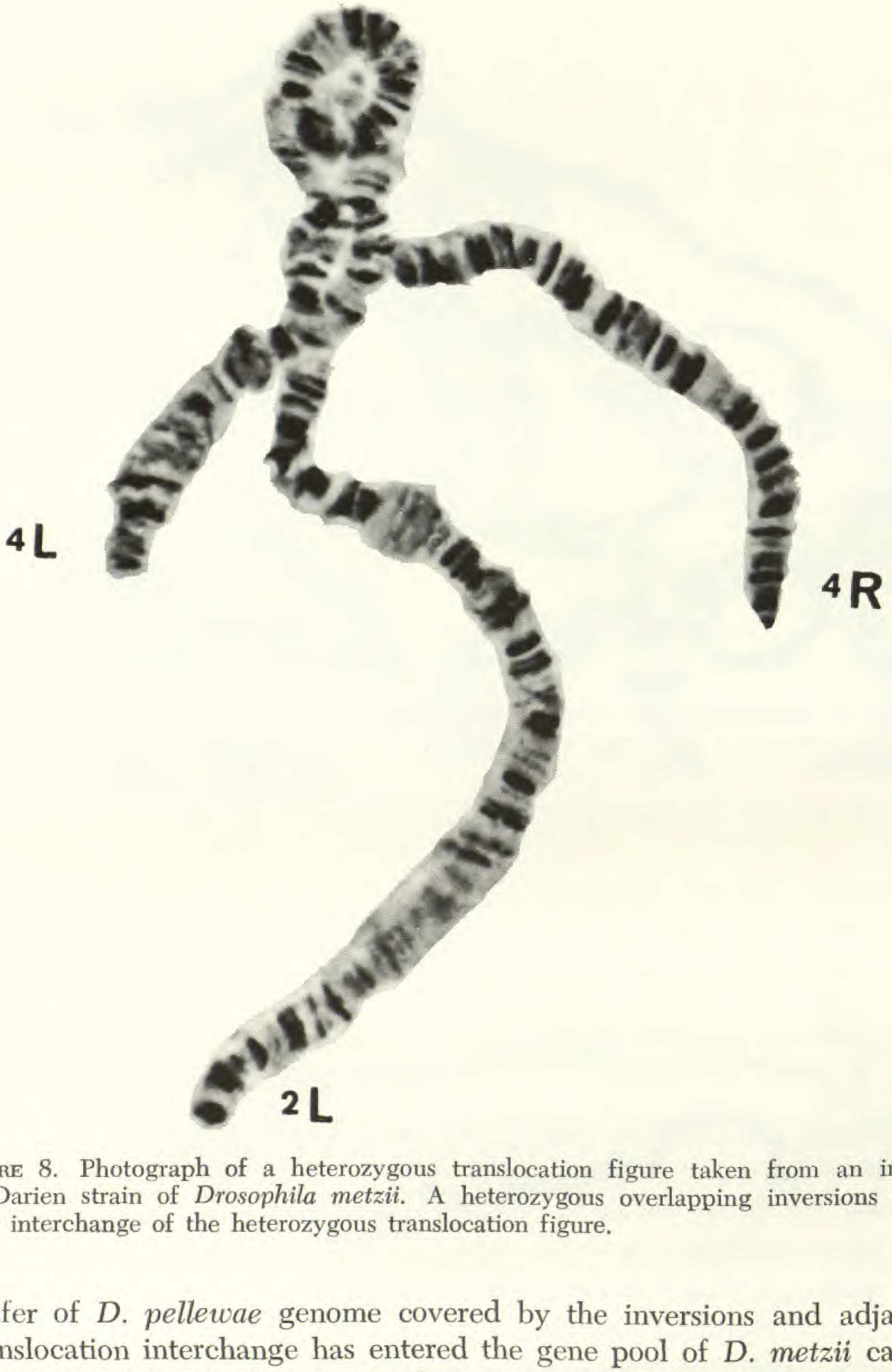

FIGURE 8. Photograph of a heterozygous translocation figure taken from an individual of the Darien strain of *Drosophila metzii*. A heterozygous overlapping inversions figure is near the interchange of the heterozygous translocation figure.

a transfer of *D. pellewae* genome covered by the inversions and adjacent to the translocation interchange has entered the gene pool of *D. metzii* causing a polymorphism. The finding in another preparation of one of the homologs of the distal portion of chromosome 5 slightly shorter than the other as in the *metzii/pellewae* hybrid of Figure 4a suggests that a translocation involving chromosome 5 is still heterozygous in the Darien *metzii* strain. In two other preparations chromosomes 2 and 5 were part of a heterozygous translocation figure involving interior regions of both chromosomes, but these cells were only sketched and not photographed. A homozygous transposition involving chromosomes 2 and 4 has been found in cells of different individuals of the Darien strain of *D. metzii* (Fig. 9A and B, with an explanatory diagram in Fig.

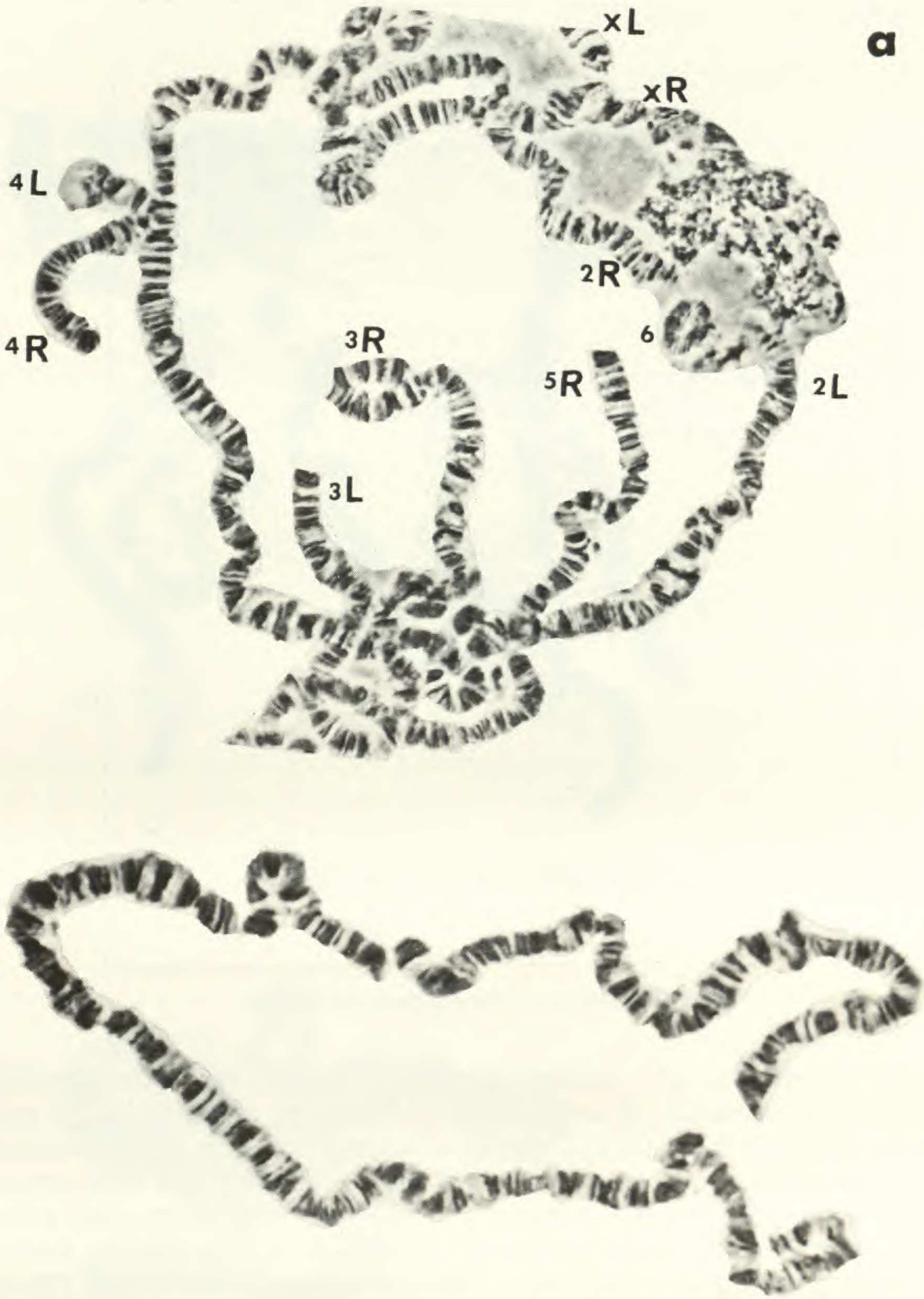

FIGURE 9. — A. Photograph of a cell of an individual of the Darien strain of *Drosophila metzii* homozygous for a 2,4 transposition with a very long "chromosome 2" and a very short "chromosome 4." — B. The long "chromosome 2" of another cell.

10). A large segment of the middle part of chromosome 4 has been inserted into the middle part of chromosome 2, with the result that the karyotype of an individual homozygous for the transposition displays an exceptionally long "chromosome 2" and a correspondingly short "chromosome 4."

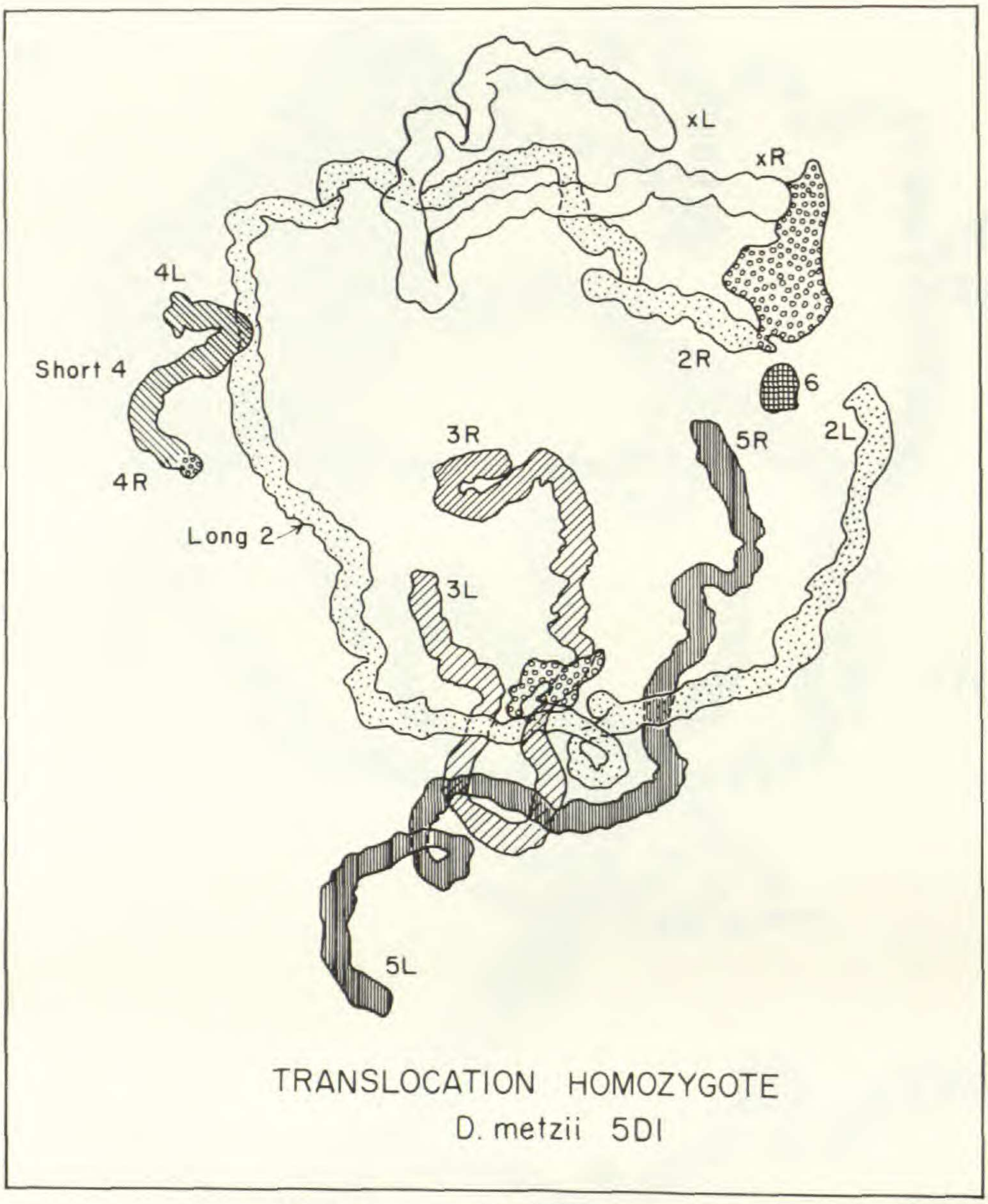

FIGURE 10. Diagram of the cell shown in Fig. 9A.

Finally introgression of a portion of *pellewae* genome into that of *metzii* is evidenced by asynapsis of certain of those homologs which failed to pair also in the species hybrid. Lack of pairing of the two homologs of the proximal part of chromosome 4 of the species hybrid shown in Figure 11A often occurs in the same region of this chromosome in the Darien *metzii* strain (Fig. 11B). Failure of pairing of the medial portion of chromosome 4 is seen in the Darien *metzii* strain occasionally (Fig. 11C). The chromosome 4 of Figure 11C is distinctly shorter than that of the standard karyotype, indicating restructuring. Asynapsis of portions of chromosome 4 in Figure 11A and B is not due to rearrangement since the banding patterns are homosequential. Even when homologs are paired, the proximal end of chromosome 4 is sometimes exceptionally broad, indicating looseness of pairing. The proximal end of the paired homologs of chromosome 2 in the Darien *metzii* strain also is sometimes broad (Fig. 11D), or even forked, indicating a slight asynapsis similar to that observed in Figures 1A, 4B, and 5 of the *metzii*/*pellewae* hybrid.

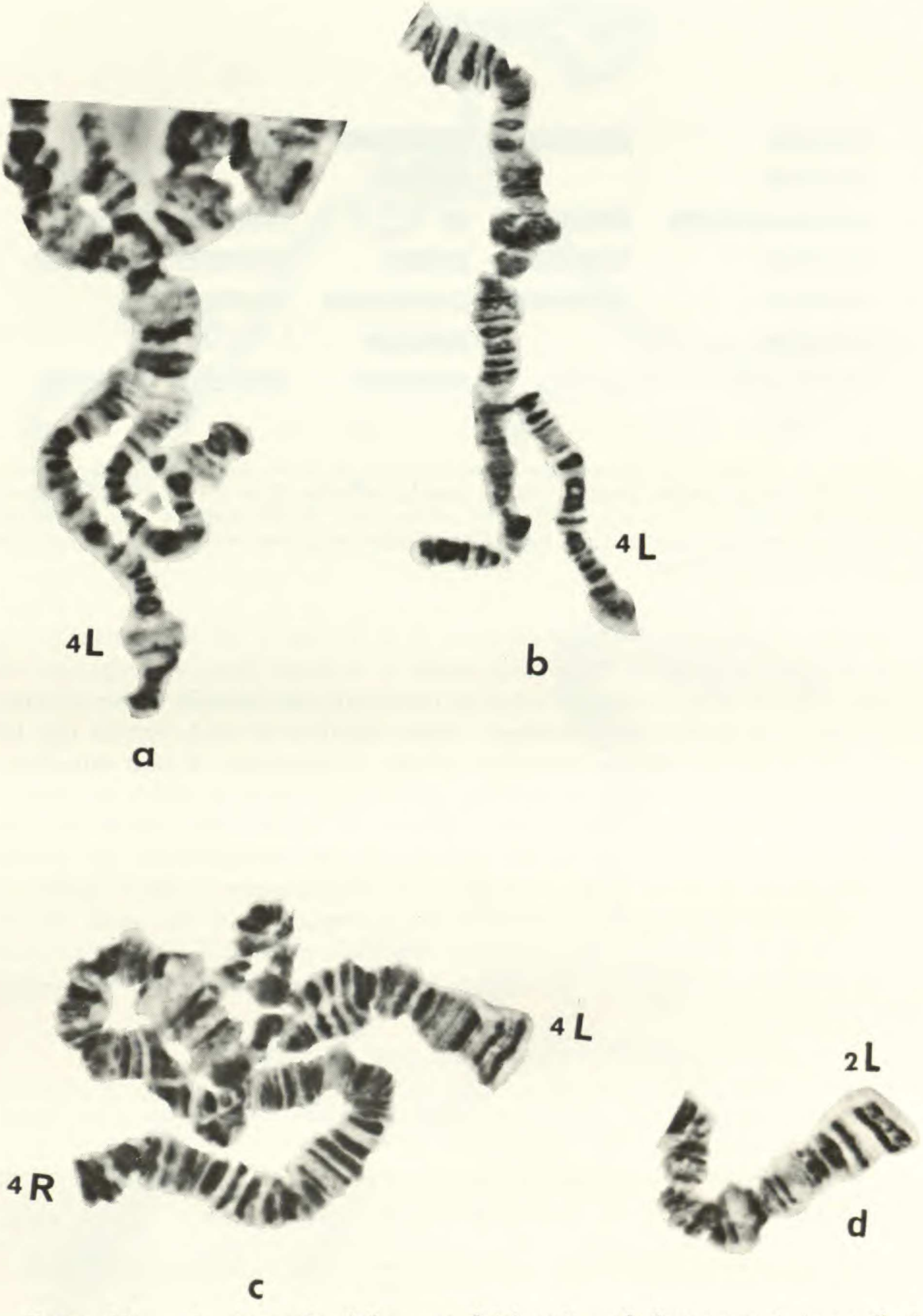

FIGURE 11. — A. Asynapsis of the same distal portion of chromosome 4 in a cell of a *Drosophila metzii*/*D. pellewae* hybrid. — B. Asynapsis of the same distal portion of chromosome 4 in a cell of the Darien strain of *D. metzii*. — C. Asynapsis of the medial portion of chromosome 4 in an individual of the Darien strain of *D. metzii*; here chromosome 4 is shorter than in the standard karyotype. — D. Broad proximal end of paired homologs of chromosome 2 in the Darien strain of *D. metzii*.

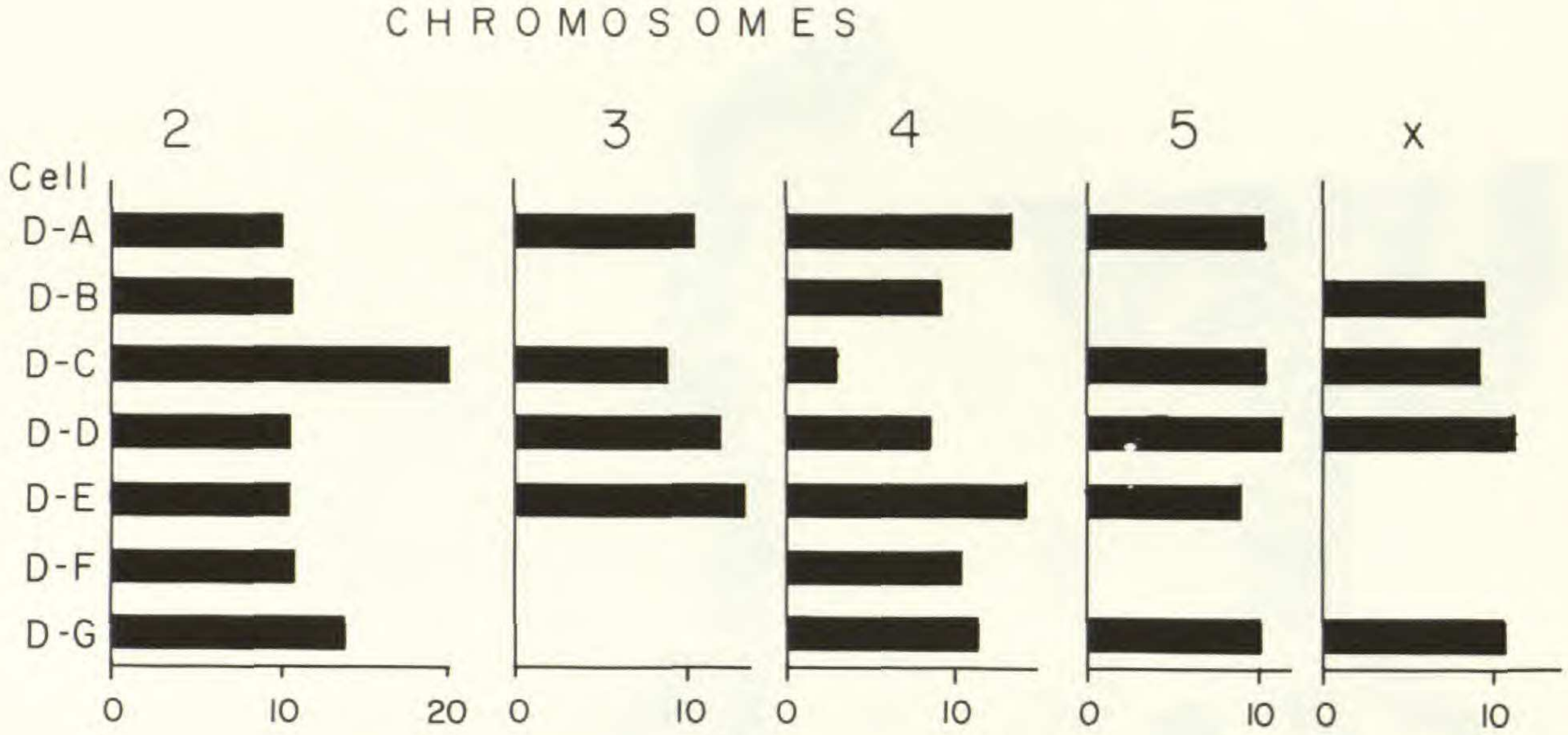

FIGURE 12. Diagram of chromosome lengths in cells of seven individuals of the Darien strain of *Drosophila metzii* showing relative lengths differing from those of the standard karyotype in which $2 > X \sim 3 > 4 > 5$. The restructuring of chromosomes is hypothesized to depend on crossing over in a *D. metzii/D. pellewae* progenitor which was heterozygous for autosomal translocations.

Further restructuring of chromosomes 2, 3, 4, and 5 of individuals taken from the Darien strain of *Drosophila metzii* is deduced from a comparison of lengths of these chromosomes obtained by measuring photographs of the chromosomes taken at similar magnifications. Some variation in such lengths can be explained as due to relative stretching of the chromosomes during squashing. To avoid this error as much as possible, cells were chosen in which the widths of paired chromosomes were similar. Figure 12 shows that except for the chromosomes of cell D-C with the homozygous 2,4 transposition, the length of chromosome 2 varied little. Variations in chromosomes 4 and 5 appeared to be inversely correlated as indicated by a comparison of the sums of the lengths of these chromosomes in the respective cells given in Table 1. Variation in the length of chromosome 3 suggests that this chromosome also has undergone restructuring.

TABLE 1. Restructuring of karyotypes suggested by comparison of lengths of respective salivary chromosomes of cells of different individuals of the Darien strain of *D. metzii*. Photographs taken at similar magnifications were used for measuring chromosomes in inches.

Chromosome(s)	2	3	4	5	X	2+4	2+4 +5	3+4 +5	2+3 +4+5
Cell D-A	10.0	10.6	13.3	10.3	—	23.3	33.6	34.2	44.2
Cell D-B	10.5	—	9.1	—	9.5	19.6	—	—	—
Cell D-C	20.0	9.1	3.0	10.5	9.2	23.0	33.5	22.6	42.6
Cell D-D	10.5	12.0	8.5	11.6	11.3	19.0	30.6	32.1	42.6
Cell D-E	10.4	13.5	14.1	9.0	—	23.9	32.9	36.6	47.0
Cell D-F	10.8	—	10.5	—	—	21.3	—	—	—
Cell D-G	13.8	—	11.3	10.1	9.8	25.1	35.2	—	—

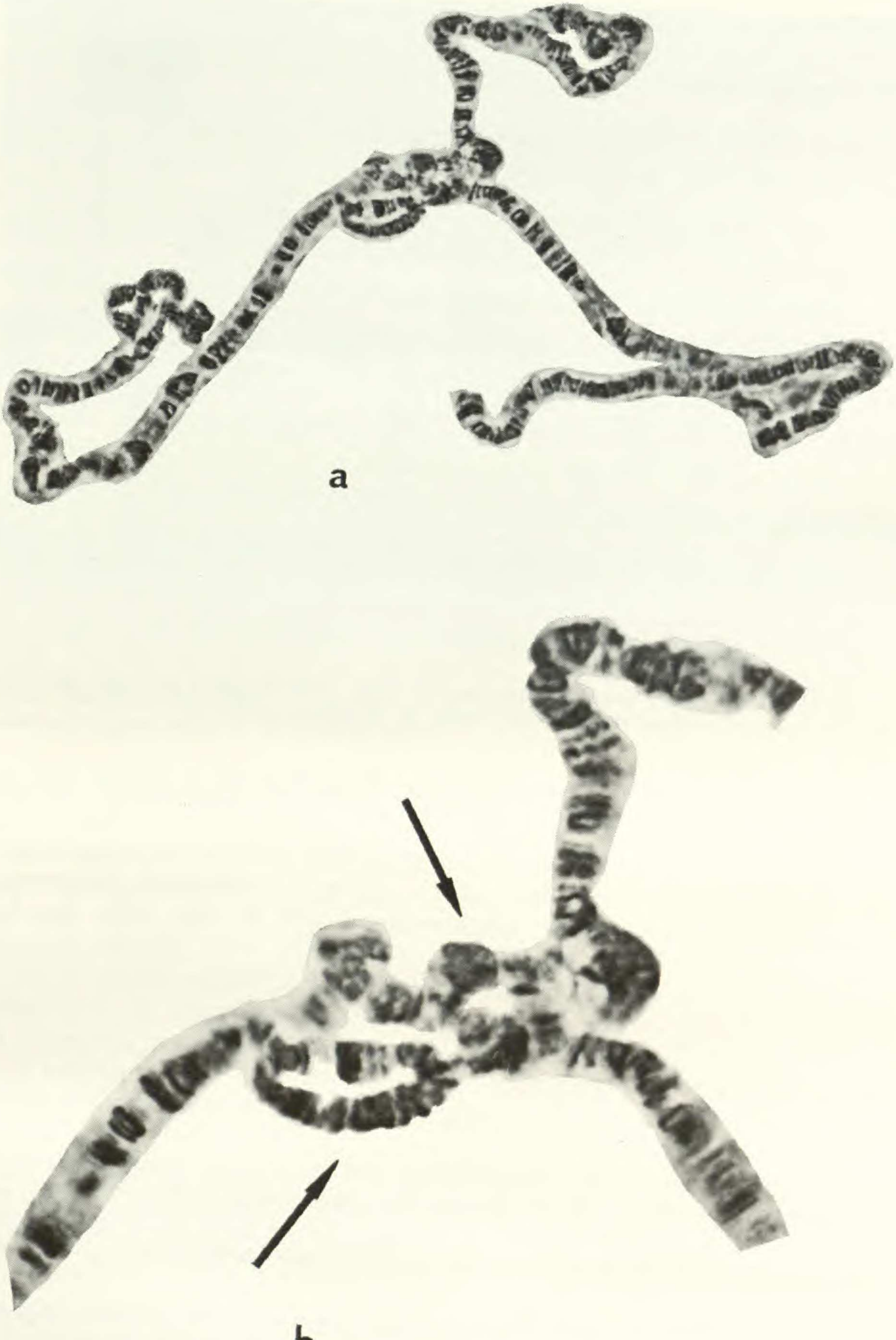

FIGURE 13. — A. Photograph of a heterozygous translocation figure taken from an individual of the Darien strain of *Drosophila pellewae*. — B. An enlargement of the region of translocation interchange showing overlapping inversions.

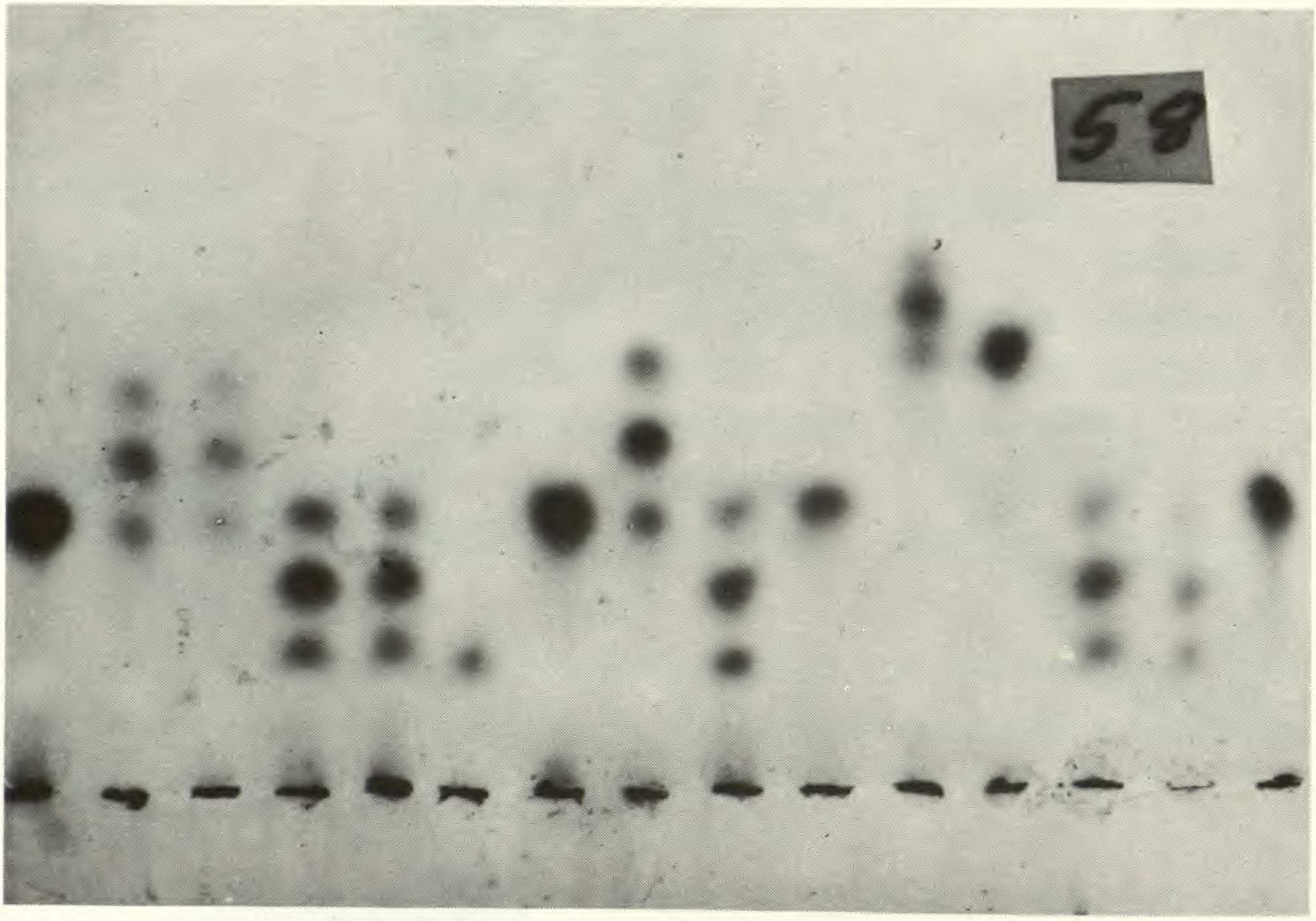

FIGURE 14. Zymograms obtained using agar gel electrophoresis and formazan staining of octanol dehydrogenase isoenzyme patterns of single adult females, 4–6 days old, taken from the unselected strain of the Darien strain of *Drosophila pellewae.*

3. Evidence of transfer of portions of the *Drosophila metzii* genome into that of *D. pellewae* of the Darien strain.

Although the salivary chromosomes of *Drosophila pellewae* are poorer material for cytological study than those of *D. metzii,* a cross-shaped heterozygous translocation figure involving chromosomes 2 and 4 (Fig. 13A) has been observed in several cells of different preparations. Two complex inversions are present at the junction of the translocation interchange shown in the enlargement of this region in Fig. 13B. The heterozygous 2,4 translocation figure found in *D. pellewae* (Fig. 13A) shows a general resemblance to that of *D. metzii* (Fig. 8), suggesting that similar portions of chromosomes 2 and 4 of each foreign species have been introgressed into the respective siblings. However, the *D. pellewae* inversion figure (Fig. 13B) indicates more extensive restructuring than in the corresponding part of the *D. metzii* heterozygous inversions near the point of translocation interchange (Fig. 8). No other heterozygous translocation figure, or asynapsis, or the large paracentric inversion of chromosome 3 has been observed in the Darien *pellewae* strain.

4. Evidence that in spite of some gene exchange, the Darien strains of *Drosophila metzii* and *D. pellewae* have remained distinct species.

An examination of the octanol dehydrogenase (ODH) isozyme patterns of the sympatric Darien strains of *Drosophila metzii* (Fig. 14) and *D. pellewae*

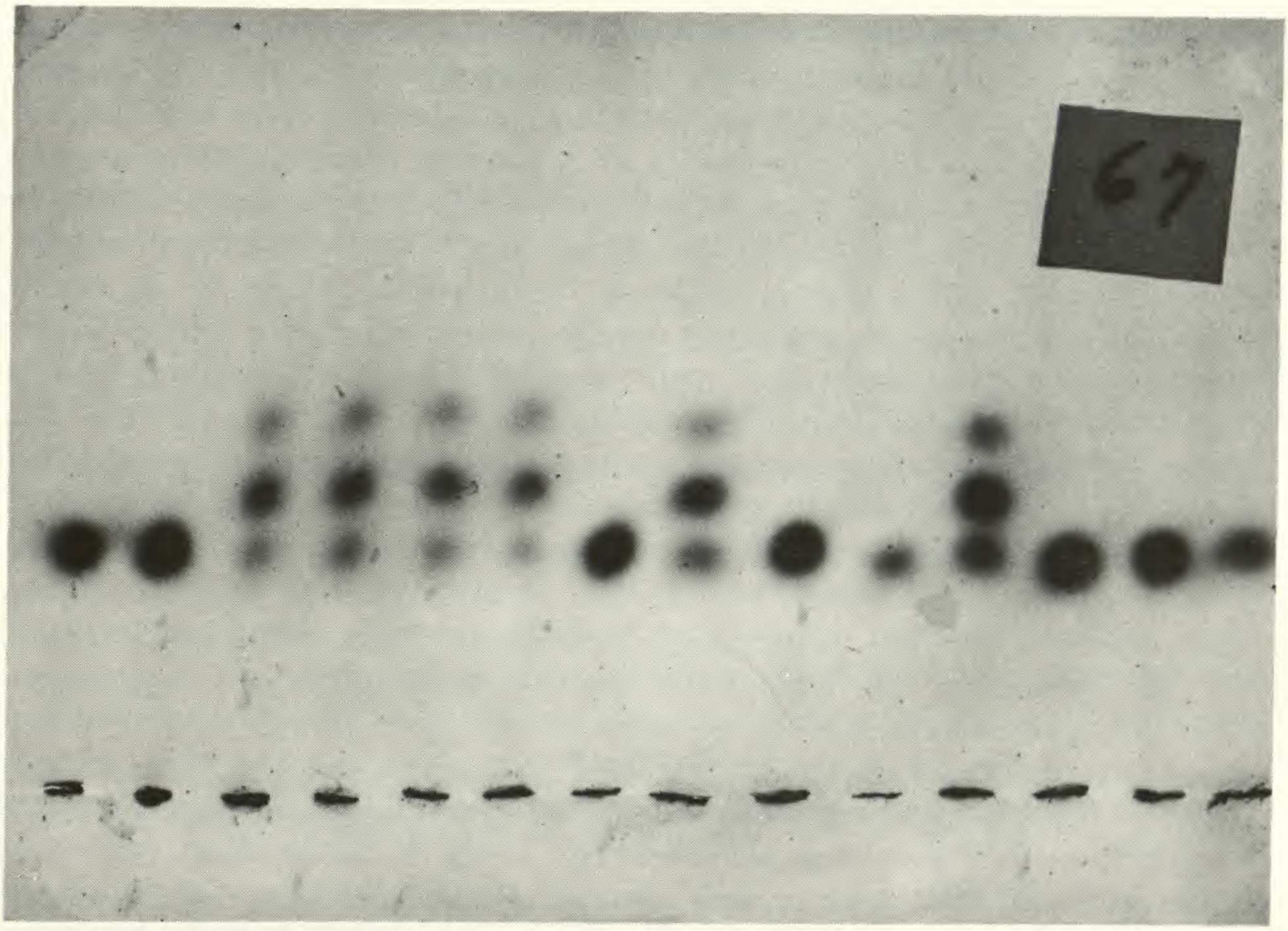

FIGURE 15. Zymograms obtained using agar gel electrophoresis and formazan staining of octanol dehydrogenase isozyme patterns of single adult females, 4–6 days old, of the unselected Darien strain of *Drosophila metzii*.

(Fig. 15) shows striking differences indicating that the genes controlling these patterns differ in the two siblings. The genetics of true breeding ODH variants isolated from these polymorphic strains has been reported by Pipkin (1968*b*, 1969). At least three structural genes coding subunits for the ODH molecule have been hypothesized by Pipkin and Bremner (1970). Homozygous variants differ in the level of certain ODH isozymes. The zymogram of mass homogenates of a strain true breeding for a given ODH pattern shows more isozymes than that of a single female of the same strain. However, breeding experiments demonstrate that all the ODH isozymes belong to the same enzyme system (Pipkin, 1968*b*, 1969). Homozygous ODH variants differ in genes regulating the synthesis of subunits coded by structural genes (or the degradation of certain isozymes). Clearly, slowly migrating isozymes can be seen in zymograms of some individuals of the Darien *pellewae* stain (Fig. 14) which cannot be detected in members of the Darien *metzii* strain (Fig. 15). Previous work has shown that the slowly migrating isozymes can be detected with formazan staining in the Darien *metzii* strain if mass homogenates are used (Pipkin & Bremner, 1970). Sympatric strains of the two sibling species thus differ in genes regulating the level of ODH isozymes.

Drosophila metzii and *D. pellewae* also differ in sexual isolation both with respect to one another and to a third sibling, *D. leticiae* (Pipkin, 1968*a*). Polygenes account for differences in sexual isolation between *D. persimilis* and *D. pseudoobscura* (Tan, 1946); between strains of *D. melanogaster* (Mather & Harrison, 1949; Petit, 1958); between members of the *D. paulistorum* complex (Ehrman, 1961); between *D. l. lebanonensis* and *D. l. casteeli* (Pipkin, 1963).

Further evidence that *Drosophila pellewae* and *D. metzii* remain distinct in the Darien Isthmus is the fact that the species can be separated by a morphological character, carina color (Pipkin & Heed, 1964). Males of *D. metzii* have a chalk white carina and face; females, a whitish carina. Males of the Barro Colorado Island strain of *D. pellewae* and females of all strains have a brown carina and face. The carina is either brown or whitish in males of the Darien *pellewae* strain. This obscure carina color polymorphism first suggested to Pipkin (1968*a*) that introgression between the two siblings was occurring. In laboratory experiments a similar obscure polymorphism was observed in progeny of *metzii/pellewae* female hybrids backcrossed with *pellewae* males. Pipkin (1968*a*) concluded that dominance modifiers which extend and intensify the white area on face and carina of *metzii* males are not present in *D. pellewae*.

DISCUSSION

Stone (1962) pointed out that restructuring of karyotypes of closely related *Drosophila* species has come about chiefly owing to inversions rather than translocations. The latter are strongly selected against because of the deleterious effect on viability of aneuploidy found among progeny of an individual heterozygous for a translocation. Three cases of translocations fixed in species were cited by Stone (1962): in *D. miranda* and *D. pseudoobscura* (Dobzhansky & Tan, 1936), in *D. ananassae* (Kaufman, 1932; Kikkawai, 1938), and in *D. tumiditarsus* (Hsiang, 1949). Translocations in the last two species and those inferred by Clayton (1969, 1971) among the Hawaiian Drosophilidae have affected mainly the amount and distribution of heterochromatin. In *D. albomicans* of the *nasuta* subgroup of the *immigrans* group, Wilson *et al.* (1969) described a fusion combining three of the five primitive rod-shaped elements characteristic of the genus *Drosophila* into one long chromosome, indicating a result of multiple translocations.

A comparison of the relative lengths of respective salivary chromosomes in nine species of the neotropical *tripunctata* species group, taken from the data of Kastritsis (1966) suggests that translocations involving *euchromatin* have played a role in restructuring the karyotypes of members of this group. The ends of chromosomes in nine species show similar banding patterns in salivary gland chromosomes, but interior parts of chromosomes are highly reorganized and cannot be homologized from species to species (Kastritsis, 1966). Measurements of chromosome maps of Kastritsis show variations in chromosome lengths among the species examined. If chromosomes are identified by the terminal banding sequences, chromosome 2 is the longest in all species except *D. mediopunctata* where it is equal in length to that of the X chromosome. Either chromosome 3 or the X is the second longest in six species, but chromosome 4 is second longest in three other species. Although chromosome 5 is the shortest in five of the nine species, it is third longest in *D. mediopictoides*. Nearly all of the *tripunctata* species possess the primitive karyotype of five pairs of rod-shaped and one pair of dot-shaped chromosomes, but fusion of chromosomes 2 and 5 and of chromosomes 3 and 4, respectively, occurs in *D. unipunctata* (Kastritsis, 1966).

A mechanism whereby a translocation, disadvantageous when heterozygous, may be maintained long enough to become homozygous, lies in the selective advantage of coadapted polygenic complexes present in the chromosomes involved in the translocation. The sparse distribution of individual *Drosophila* species in neotropical forests (Pipkin, 1965) must contribute to the isolation and thus persistence of populations in which translocations become fixed. Isolation by translocation homozygosity could contribute ultimately to a sympatric differentiation of species. The importance of coadapted polygenic complexes in the maintenance of inversion heterozygosity has been discussed by Levitan (1958), Kastritsis (1966), and Carson (1969). The present study on Darien populations of *D. metzii* and *D. pellewae* suggests that individuals homozygous for a transposition arise as recombinants in descendants of a *metzii/pellewae* hybrid, similar to the crossover products described by McDonald and Rai (1970) in the progeny of double translocation heterozygotes of *Aedes aegypti*. Even when *D. metzii/D. pellewae* hybrids are produced occasionally in nature, coadaptation of the entire chromosome set of each species leads usually to the continued isolation of these sibling species. However, an opportunity for limited gene exchange due to crossing over is afforded. Since *metzii/pellewae* hybrid males are sterile, gene exchange must occur through backcrossing rare hybrid females with males of either parental species, *i.e.* through introgression.

Although *Drosophila metzii* and *D. pellewae* are difficult cytological material, they present a challenge for the future to discover the extent of karyotype restructuring in each species resulting from introgression in the Darien overlap zone.

LITERATURE CITED

ANDERSON, E. 1949. Introgressive Hybridization. New York.

CARSON, H. L. 1969. Parallel polymorphisms in different species of Hawaiian *Drosophila*. Amer. Naturalist 103: 323–329.

CLAYTON, F. E. 1969. Variations in the metaphase chromosomes of Hawaiian *Drosophilidae*. Studies in genetics V. Univ. Texas Bull. 6918: 95–110.

———. 1971. Additional karyotypes of Hawaiian *Drosophilidae*. Studies in genetics VI. Univ. of Texas Bull. 7103: 171–181.

DOBZHANSKY, T. & C. C. TAN. 1936. Studies in hybrid sterility III. A comparison of the gene arrangement in two species of *Drosophila*. Z.I.A.V. 72: 88–114.

EHRMAN, L. 1961. The genetics of sexual isolation in *D. paulistorum*. Genetics 46: 1025–1038.

HEED, W. B. & M. R. WHEELER. 1957. Thirteeen new species in the genus *Drosophila* from the neotropical region. Univ. Texas Publ. 5721: 17–38.

HSIANG, W. 1949. The distribution of heterochromatin in *D. tumiditarsus*. Cytologia 15: 149–152.

KAUFMAN, B. P. 1932. Morphology of the chromosomes of *D. ananassae*. Pp. 1043–1055 *in* Cytologia Fujii Jubilee Vol.

KASTRITSIS, C. D. 1966. Cytological studies on some species of the *tripunctata* group of *Drosophila*. Studies in genetics III. Univ. Texas Publ. 6615: 413–474.

KIKKAWAI, H. 1938. Studies on the genetics and cytology of *D. ananassae*. Genetica 20: 458–516.

LEVITAN, M. 1958. Non-random associations of inversions. Cold Spring Harbor Sympos. Quant. Biol. 23: 251–268.

McDONALD, P. T. & K. S. RAI. 1970. *Aedes aegypti*: Origin of a "new" chromosome from a double translocation heterozygote. Science 168: 1229–1230.

MATHER, K. & B. J. HARRISON. 1949. The manifold effect of selection. Heredity 3: 1–52.

PETIT, C. 1958. Le determinisme génètique et psycho-physiologique de la competition sexuelle chez *D. melanogaster*. Bull. Biol. France Belgique 92(3): 1–82.

PIPKIN, S. B. 1963. Mating success in the *Drosophila victoria* species group, subgenus *Pholadoris* (Diptera: Drosophilidae). Amer. Naturalist 97: 235–242.

———. 1965. The influence of adult and larval food habits on population size of neotropical ground-feeding *Drosophila*. Amer. Midl. Naturalist 74: 1–27.

———. 1968*a*. Introgression between closely related species of *Drosophila* in Panama. Evolution 22: 140–156.

———. 1968*b*. Genetics of octanol dehydrogenase in *Drosophila metzii*. Genetics 60: 81–92.

———. 1969. Genetic evidence of a tetramer structure of octanol dehydrogenase of *Drosophila*. Genetics 63: 405–418.

——— & T. A. BREMNER. 1970. Aberrant octanol dehydrogenase isozyme patterns in interspecific *Drosophila* hybrids. Jour. Exp. Zool. 175(3): 283–295.

——— & W. B. HEED. 1964. Nine new members of the *Drosophila tripunctata* species group (Diptera: Drosophilidae). Pacific Insects 6: 256–273.

STEBBINS, G. L. 1950. Variation and Evolution in Plants. New York.

STONE, W. S. 1962. The dominance of natural selection and the reality of superspecies (species groups) in the evolution of *Drosophila*. Univ. Texas Publ. 6205: 507–537.

TAN, C. C. 1946. Genetics of sexual isolation between *D. pseudoobscura* and *D. persimilis*. Genetics 31: 558–573.

WASSERMAN, M. 1954. Cytological studies of the *repleta* group. Univ. Texas Publ. 5422: 130–152.

WILSON, F. D., M. R. WHEELER, M. HARGET & M. KAMBYSELLIS. 1969. Cytogenetic relations in the *Drosophila nasuta* subgroup of the *immigrans* group of species. Studies in genetics V. Univ. Texas Publ. 6918: 207–253.